PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	REducing STEroids in Relapsing Nephrotic syndrome: the RESTERN study – protocol of a national, double-blind, randomised, placebo controlled, noninferiority intervention study
AUTHORS	Schijvens, A; Dorresteijn, E; Roeleveld, C; ter Heine, R; van Wijk, J; Bouts, A; Keijzer - Veen, M; van de Kar, N; van den Heuvel, L; Schreuder, Michiel

VERSION 1 – REVIEW

REVIEWER	Professor Dr. Lutz T. Weber Head of Pediatric Nephrology Children`s and Adolescents´ Hospital University Hospital of Cologne Kerpener Str. 62 50937 Cologne Germany
REVIEW RETURNED	21-Jun-2017

GENERAL COMMENTS	The RESTERN study will not only contribute but expand the therapeutic knowledge on steroid sensitive idiopathic nephrotic syndrome in children. This non-inferiority study takes up the most important issue on reduction of steroid exposure and toxicity. The idea is to contract treatment duration of alternate day prednisolone from 6 to 2 weeks in relapsing nephrotic syndrome. The initiators of RESTERN are to be congratulated on this next randomized prospective study in pediatric nephrotic syndrome in the Netherlands. Being a rare disease makes prospective randomized studies with fair power extremely difficult. The Dutch pediatric nephrologists have, however, only recently shown that they are able to motivate the entire country to recruit nearly every patient eligible (7). The Dutch standard treatment of relapse provides 6 weeks of prednisolone on alternate days. This approach differs from the recommendation of KDIGO that proposes prednisone on alternate days for “at least 4 weeks). The authors adequately discuss this issue with respect to its rationale. Furthermore, in case non-inferiority of the study approach is being shown the results should also be transferrable to the KDIGO recommendation of 4 weeks. Anyway the approach to shorten the exposure to prednisolone is very well taken. Indeed prospective studies on this issue are lacking and we have learned from well performed prospective studies on the initial treatment of idiopathic nephrotic syndrome in childhood (20,21) that duration of and exposure to steroids do not influence the natural course of the disease (17). Strictly taken prednisolone is not equal to prednisone, but results in higher glucocorticoid load when given in the same dose due to its pharmacokinetic characteristics.

	In general, however, it is regarded as clinically equivalent. The authors decided to also include patients receiving maintenance immunosuppressive therapy. This decision may limit the study results. One can assume that patients on maintenance immunosuppressive therapy do have a complicated course of the disease and are probably frequent relapsers or steroid dependent. Not only they suffer from a more aggressive course of nephrotic syndrome and may not be comparable to infrequent relapsers but also immunosuppression does influence the course of the disease with complete avoidance of relapse at best. It will be important to analyze patients with and without maintenance immunosuppression separately, as they reflect different patient groups. One can hypothesize that maintenance immunosuppression may compensate for a reduction of alternate day prednisolone. It is correct to randomize these two patient groups separately as the protocol plans. Unfortunately, the aspect of “two studies in one” is not regarded in the calculation of sample size. One may scrutinize the effort to double-blind the study. The study medication is characterized by an overt and distinct toxicity, whose absence or decline may uncover blinding clinically. It is remarkable that cumulative steroid dose is assessed in the study but potential steroid associated toxicity is not, being the overt rationale to reduce steroid exposure in idiopathic nephrotic syndrome in children after all. The authors describe the primary outcome of the RESTERN study as “time to first relapse. This is defined as the time (in days) from the final prednisolone dose until the first day of treatment of the next relapse”. Since this study is randomized and double-blinded it should be “study medication” instead of “prednisolone” in the definition. Of note the definition does not include potential relapses within the treatment period. Participants should have the urine dipstick done every day instead of “at least every other day” to determine remission. Any other schedule than every day may prolong and diversify the exposure to daily prednisolone. This may influence the study results. The sample size has been calculated to be 72 patients per group and the corresponding time of recruitment is supposed to be 1.5 years. The paper announces 270 children with nephrotic syndrome and relapse each year in the age range from 2 to 12 years. According to the incidence of idiopathic nephrotic syndrome in the Netherlands (1.5/100.000 children) and assuming a rate of 80% to develop a relapse one would, however, end up with a number rather close to 120 children with relapse each year. Nevertheless, the historic experience shows that the motivation and recruitment activity of the Dutch centers is excellent. Therefore the aim to include the patient number aimed at within 1.5 years does not seem to be impossible. However, I have not seen any correction for potential screening failures, drop-outs and losses of follow-up in the manuscript. It is certainly both, necessity and strength of the study to have a data safety monitoring board (DSMB) and to perform an interim analysis. To avoid split decisions one would rather have composed the DSMB of an uneven number of members. Despite of these comments pediatric nephrologists are keen on coming to know the results of the RESTERN study.

REVIEWER	Arvind Bagga All India Institute of Medical Sciences, New Delhi, India Developing & implementing investigator initiated, single- or multicenter prospective clinical trials on children with nephrotic syndrome
REVIEW RETURNED	29-Jun-2017

GENERAL COMMENTS	This study addresses an important question regarding therapy for relapses of nephrotic syndrome. The study is proposed by a group with highly satisfactory experience & expertise in conducting similar studies. In a double blind, randomized controlled, nationwide trial the authors intend to compare the effectiveness of 2-week to 6-week alternate day therapy. Stratification is proposed, based on presence or absence of additional immunosuppressive agents. Major points An issue, that is also mentioned by the authors, is that most specialists across the world would treat relapses with 4- instead of 6-weeks alternate day therapy. This might also be the standard of care in Netherlands. Are we really comparing two new therapies in this RCT, and would it not have been more relevant to compare 4-weeks (standard) to 2-weeks (new) treatment instead. 1. The objectives are clearly stated although there is some discordance in the way they are mentioned in some portions of the text. The primary objective is the time to first relapse. Secondary objectives are: the number of relapses, progression to frequent relapses or dependence, and cumulative prednisone intake at 12- and 24-months follow up. In some places this is stated slightly differently, and would require to be harmonized. 2. If subgroup analyses is planned to separately examine effectiveness in patients receiving concomitant non-steroidal immunosuppressive therapies (as mentioned at some places) the sample size would need to increase appropriately. 3. Is randomization proposed to be central or center-specific? 4. The point of randomization is the point from which follow up begins. The point should NOT be 4 weeks later in the standard therapy group. 5. Might have missed, but what will be the frequency of visits during 24 months follow up? And how frequently will parents be doing dipstick exam at home during follow up? 6. Sample size: In calculating sample size, the time to first relapse of 185 days seems rather long, especially if one considers that there will also be children with frequent relapses/dependence where this duration might be briefer. Does it also take into account that interim analysis is planned? If subgroup analyses is planned, this would result in revision of sample size. Looking at the study design, this in fact should be considered, similar to what is happening in the PREDNOS 2 study. 7. Given that the overall aim is to reduce corticosteroid therapy and since there is a 24-month follow up, it might be useful to state upfront that the study will (as a secondary objective) also compare features of steroid toxicity, as also height/weight/BMI SDS.

VERSION 1 – AUTHOR RESPONSE

Comments reviewer 1:

Comment 1:The Dutch standard treatment of relapse provides 6 weeks of prednisolone on alternate days. This approach differs from the recommendation of KDIGO that proposes prednisone on alternate days for “at least 4 weeks”. The authors adequately discuss this issue with respect to its rationale. Furthermore, in case non-inferiority of the study approach is being shown, the results should also be transferrable to the KDIGO recommendation of 4 weeks.

Response: Thank you for your comment regarding the transferability of our results. As most pediatricians in the Netherlands follow the Dutch treatment guidelines, it was most convenient to consider the 6 week alternate day prednisolone regimen as the standard treatment group. We agree with the reviewer that in case noninferiority is shown, our results are indeed transferrable to the KDIGO recommendation. In case inferiority is shown, additional research is needed.

Comment 2: The decision to also include patients receiving maintenance immunosuppressive therapy may limit the study results. One can assume that patients on maintenance immunosuppressive therapy do have a complicated course of the disease and are probably frequent relapsers or steroid dependent. Not only they suffer from a more aggressive course of nephrotic syndrome and may not be comparable to infrequent relapsers, but also immunosuppression does influence the course of disease with complete avoidance of relapse at best. It is correct to randomize these two patient groups separately as the protocol plans. Unfortunately, the aspect of “two studies in one” is not regarded in the calculation of sample size.

Response: Thank you, this point is well taken. This issue has indeed been discussed extensively in the process of designing our study. We absolutely agree with the reviewer that patients with immunosuppressive maintenance therapy will most likely have a more complicated disease course. However, as the treatment regimen for a nephrotic syndrome relapse is the same for patients with and without maintenance therapy, we chose to include both groups and base our primary outcome on the total population. This way we hope to make our study findings as broadly generalizable as possible. As the reviewer states above, we indeed stratify for maintenance therapy in the randomization process. Subgroup analysis will be performed for patients with and without maintenance therapy. Conclusions regarding the separate groups might be inconclusive as the study is not powered to detect differences in treatment effect across these subgroups. These analyses will be considered as hypothesis generating.

Comment 3: It is remarkable that cumulative steroid dose is assessed in the study, but potential steroid associated toxicity is not, being the overt rationale to reduce steroid expose in idiopathic nephrotic syndrome in children.

Respobnse: Thank you for this comment, we apologize that this was not properly explained in our manuscript. The digital questionnaires include questions about side effects and steroid toxicity at different time points, e.g. during the period of daily prednisolone, alternate day prednisolone and study medication. Topics assessed include: infections, cushingoid appearance, nausea, psychological problems, eye problems, increased appetite, muscle weakness, hypertension etc. First, patients are asked whether the side effect is/was present during the specific period. Second, the level of inconvenience is assessed (ranging from not at all inconvenient to very inconvenient). In addition, patients are asked whether hospital admission was required. We added this information to the manuscript in the data collection section on page 10-11. In addition, the effect of the reduced treatment schedule on bone mineral density will not be assessed in this study.

We enclosed one of the questionnaires as an example, in which we highlighted the questions regarding side effects and steroid toxicity. The questionnaire is in Dutch, however using the yellow marking we want to indicate the questions related to side effects and steroid toxicity.

Comment 4: The authors describe the primary outcome of the RESTERN study as “time to first relapse”. This is defined as the time (in days) from the final prednisolone dose until the first day of treatment of the next relapse. Since this study is randomized and double-blinded, it should be study medication instead of prednisolone in the definition. Of note: the definition does not include potential relapses within the treatment period.

Response: We feel it is most appropriate to assess the time to first relapse calculated from the day after the final prednisolone dose instead of the start of study medication, as this comes down to a difference of 4 weeks between the placebo and standard treatment group. However, we are aware of the aforementioned issue. Therefore, all final statistical analysis will be performed after unblinding, as stated in the statistical analysis plan on page 3. In case a relapse occurs during the period of study medication, the duration of study medication is assessed and after unblinding the time to relapse is calculated.

Comment 5: Participants should have the urine dipstick done every day instead of “at least every other day” to determine remission. Any other schedule than every day may prolong and diversify the exposure to daily prednisolone. This may influence the study results.

Response: Thank you for this comment and this valuable recommendation. Parents are indeed instructed to perform dipstick analysis every day. This change is carried through in our manuscript (page 8).

Comment 6: The sample size has been calculated to be 72 patients per group and the corresponding time of recruitment is supposed to be 1.5 years. The paper announces 270 children with nephrotic syndrome and relapse each year in the age range from 2 to 12 years. According to the incidence of idiopathic nephrotic syndrome in the Netherlands (1.5/100.000 children) and assuming a rate of 80% to develop a relapse one would, however, end up with a number rather close to 120 children with relapse each year. Nevertheless, the historic experience shows that the motivation and recruitment activity of the Dutch centers is excellent. Therefore the aim to include the patient number aimed at within 1.5 years does not seem to be impossible. However, I have not seen any correction for potential screening failures, drop-outs and losses of follow-up in the manuscript.

Response: Thank you for your confidence regarding the recruitment of patients with nephrotic syndrome in the Netherlands. Our calculation was slightly different from the calculation of the reviewer: we based the number of children on the fact that approximately 180,000 children are born each year. With a population at risk between the ages of 2 and 12 years and a prevalence of 15/100,000, about 270 children may be at risk of developing a nephrotic syndrome relapse.

- Subjects will be replaced after withdrawal. Based on withdrawal rates of a previous nephrotic syndrome clinical trial in The Netherlands (Teeninga et al., 2013) a maximum of 23 subjects (=16%) will be replaced. The reason for withdrawal will be recorded in their medical status report and the trial master file. This information is added to the sample size section in the manuscript. (Page 9)

Comment 7: It is certainly both, necessity and strength of the study to have a data safety monitoring board (DSMB) and to perform an interim analysis. To avoid split decisions one would rather have composed the DSMB of an uneven number of members.

Response: Thank you for this valuable comment. We agree that it may have been more convenient to compose the DSMB of three members. We will take this into account in future studies. The aim for the DSMB for the RESTERN study is to check for a significant difference in relapse rate between the two groups based. We made clear guidelines and cut-off points regarding this significant difference. Therefore, split decisions are considered unlikely in this case.

Comments reviewer 2:
Comment 1: An issue, that is also mentioned by the authors, is that most specialists across the world would treat relapses with 4- instead of 6-weeks alternate day therapy. This might also be the standard of care in Netherlands. Are we really comparing two new therapies in this RCT, and would it not have been more relevant to compare 4-weeks (standard) to 2-weeks (new) treatment instead.

Response: Thank you for this valuable comment. We indeed addressed the issue in the Dutch/Belgian national Working group for Idiopathic Nephrotic Syndrome (WINS), in which all academic hospitals are represented.
As the KDIGO recommendation of at least 4 weeks alternate day steroid therapy was based on a rather small study, in the Netherlands the 6 week alternate day schedule described by the Arbeitsgemeinschaft für Paediatrische Nephrologie (APN) is traditionally used. This schedule is therefore recommended in the Dutch Pediatric Nephrology handbook (ISBN 978 90 8659 462 7, 2010, VU University Press, Amsterdam). As most pediatricians in the Netherlands follow the practice guidelines proposed in this handbook, we chose to compare the 6-week to 2-week treatment. In case noninferiority is shown, our results are also transferrable to the KDIGO recommendation of at least four weeks. However, in case inferiority is shown, additional research is needed.

Comment 2: The objectives are clearly stated although there is some discordance in the way they are mentioned in some portions of the text. The primary objective is the time to first relapse. Secondary objectives are: the number of relapses, progression to frequent relapses or dependence, and cumulative prednisone intake at 12- and 24-months follow up. In some places this is stated slightly differently, and would require to be harmonized.

Response: Thank you for drawing our attention to this inconsistency. Changes in the abstract (page 2) are made accordingly.
We deliberately chose to define both study objectives and study outcomes. Study outcomes (page 8), e.g. time to next relapse, number of relapses, progression to frequent relapsing or steroid dependent nephrotic syndrome and cumulative dosage of prednisolone, will be used to answer the questions raised in the study objectives (page 6).

Comment 3: If subgroup analyses is planned to separately examine effectiveness in patients receiving concomitant non-steroidal immunosuppressive therapies (as mentioned at some places) the sample size would need to increase appropriately.

Response: Thank you, this issue has indeed been discussed extensively in the process of designing our study.
We have based the sample size on the primary outcome: time to first relapse after the final prednisolone dose. The group of patients experiencing a relapse of nephrotic syndrome is heterogeneous. To make our study findings as broadly generalizable as possible, we chose to include subjects with and without immunosuppressive maintenance therapy. As maintenance therapy can influence the time to next relapse, we stratify for this in the randomization procedure. However, as treatment of a nephrotic syndrome relapse is the same for patients with and without maintenance therapy, our primary outcome is based on the total population. Therefore, we believe the sample size to be adequate. Subgroup analysis will indeed be performed for patients with and without maintenance therapy; conclusions regarding the separate groups might be inconclusive as the study

is not powered to detect differences in treatment effect across these subgroups. These analyses will be considered as hypothesis generating.

Comment 4: Is randomization proposed to be central or center-specific?

Response: The randomization is performed centrally. Due to the small numbers, local hospitals are likely to include 1-2 participants, therefore randomization and coordination of the study is performed centrally as indicated in the section 'Randomisation and blinding' on page 10 of the study protocol.

Comment 5: The point of randomization is the point from which follow up begins. The point should NOT be 4 weeks later in the standard therapy group.

Response: The follow-up period of 24 months will indeed start at the time of randomization (start of study medication). However, the primary outcome, time to first relapse, will be assessed from the day of the final prednisolone dose. This implicates a difference of 4 weeks between the standard treatment and placebo group. Due to the double-blind character of this study, time to first relapse and thereby all final statistical analyses can only be determined after unblinding.

Comment 6: Might have missed, but what will be the frequency of visits during 24 months follow up? And how frequently will parents be doing dipstick exam at home during follow up?

Response: The frequency of visits is indeed not specified in our study protocol. After the study medication, follow-up of the patients will be via digital questionnaires at different time points, as shown in table 2 (page 9). Hospital visits are arranged with the treating pediatrician or pediatric nephrologist at their own discretion. Our primary and secondary outcomes are independent of hospital visits. On average patients will visit the clinic 2-4 times each year, depending on the course of disease. During a follow-up visit, patients undergo physical examination (including measurement of blood pressure, assessing weight/height/BMI) and laboratory investigation (including serum albumin, creatinine, blood urea nitrogen, cholesterol, urine protein levels and urine protein-to-creatinine ratio). Dipstick analysis will be performed by the parents at their own discretion, e.g. if any changes in the appearance of their child is present and in case of infection or other diseases state.

Comment 7: Sample size: In calculating sample size, the time to first relapse of 185 days seems rather long, especially if one considers that there will also be children with frequent relapses/dependence where this duration might be briefer. Does it also take into account that interim analysis is planned? If subgroup analyses is planned, this would result in revision of sample size. Looking at the study design, this in fact should be considered, similar to what is happening in the PREDNOS 2 study.

A previous trial in the Netherlands performed by Teeninga et al (2013), showed that the average time to first relapse was 185 days. Unfortunately, data about time to subsequent relapses is lacking. We agree with the reviewer that in our patient group the time to next relapse will most likely be shorter as we also include patients with frequent relapsing nephrotic syndrome. On the other hand, with adequate immunosuppressive maintenance therapy to prevent subsequent relapses, the time to next relapse may also be longer. Therefore, we decided to maintain the time to next relapse of 185 days.

Response: We would like to thank the reviewer for the suggestion to revise our sample size similar to the PREDNOS 2 study. After careful consideration, we decided that this will not be applicable to the RESTERN study as subgroup analysis will only be performed in the final analysis and in the interim analysis time to relapse will not be assessed. The interim analysis, after a three month follow-up of the first 40 patients, will be performed by the Data Safety and Monitoring Board.

Aim is to check for a significant difference in relapse rate between the two groups. Initially, this will be evaluated using dummy groups (group A and group B). Only if there seems to be a significant difference between the two groups and the percentage of patients with a relapse in either one or both groups exceeds 40% than they might consider to unblind the groups.

In the final statistical analysis, subgroup analysis will be performed for patients with and without maintenance therapy. Hopefully, we can provide some answers regarding the subgroups, however, it is also possible that additional research is needed and our conclusions regarding the subgroups will be considered hypothesis generating.

Comment 8: Given that the overall aim is to reduce corticosteroid therapy and since there is a 24-month follow up, it might be useful to state upfront that the study will (as a secondary objective) also compare features of steroid toxicity, as also height/weight/BMI SDS.

Response: Excellent point, we apologize that this was not properly explained in our manuscript. In the digital questionnaires steroid toxicity is indeed assessed. Topics assessed include: infections, cushingoid appearance, nausea, psychological problems, eye problems, increased appetite, muscle weakness, hypertension etc. First, patients are asked whether the side effect is/was present during the specific period. Second, the level of inconvenience is assessed (ranging from not at all inconvenient to very inconvenient). In addition, patients are asked whether hospital admission was required. We added this information to the manuscript in the data collection section. In addition, height/weight and BMI are also assessed in these questionnaires and in the medical records provided by the local pediatricians and pediatric nephrologists.
We enclosed one of the digital questionnaires as an example, in which we highlighted the questions regarding side effects and steroid toxicity. We apologize for the Dutch language.

VERSION 2 – REVIEW

REVIEWER	Professor Dr. Lutz T. Weber Children´s and Adolescents´ Hospital, University Hospital of Cologne, Germany
REVIEW RETURNED	29-Aug-2017

GENERAL COMMENTS	Thank you for the careful revision of your study protocol. I think we agree about its limitations. Nevertheless this study will contribute to and increase current knowledge. I do hope the authors will meet their ambitious target of recrutation. We are keen on coming to know the results in the future. Good luck!

REVIEWER	Arvind Bagga All India Institute of Medical Sciences India
REVIEW RETURNED	10-Sep-2017

GENERAL COMMENTS	The authors have addressed the comments adequately.